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STUDIES ON OXYGEN TOXICITY IN THE LUNGS.(U)
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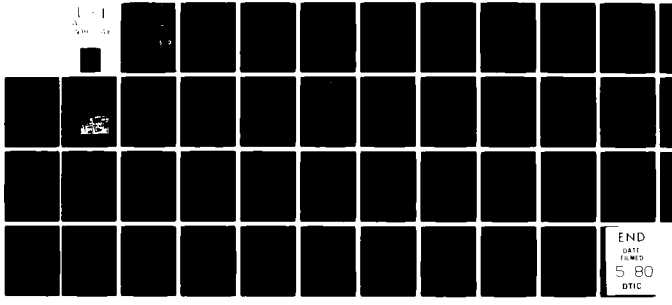

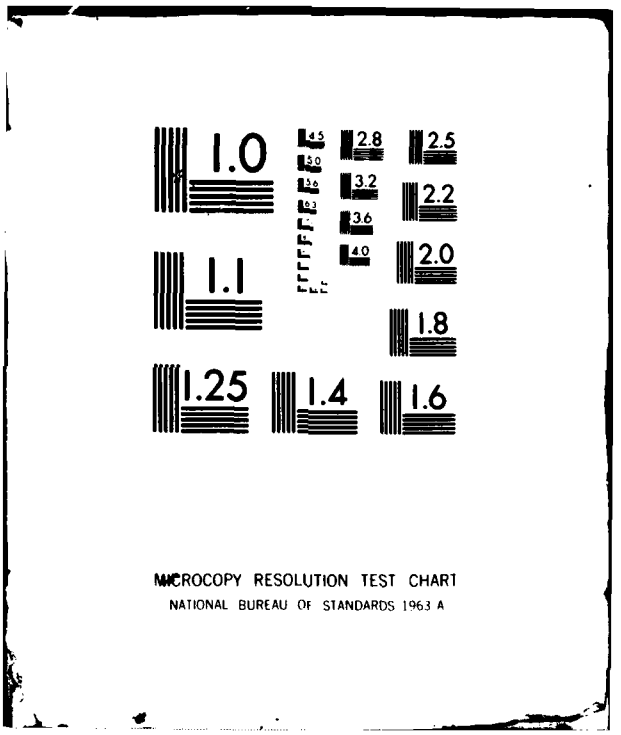
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Work on two animal species ~~under this contract~~ has shown that during the first day of exposure to high concentrations of oxygen (O_2), the normal rate of cell division in the lung is decreased. In animals that survive the initial exposure to O_2 or in those exposed to a lower concentration, cell division resumes after about five days.

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20. ABSTRACT (Continued)

In animals exposed to O_2, it has been noted that they eat less and lose weight. ~~We have shown that~~ air-breathing mice deprived of food had weight loss and depression in DNA synthesis similar to that observed in mice exposed to 95 percent O_2. These results indicate that indirect factors are associated with the depression of DNA synthesis observed in mice exposed to high O_2.

~~We have also shown that~~ lack of food or 40 percent O_2 did not inhibit type 2 division associated with repair of ozone damaged alveolar epithelium. Oxygen concentrations of 61 percent or higher did inhibit reparative cell division in ozone damaged alveoli of mice.

FINAL TECHNICAL REPORT OFFICE OF NAVAL RESEARCH

STUDIES ON OXYGEN TOXICITY IN THE LUNGS

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A. OVERVIEW

Work on two animal species under this contract has shown that during the first day of exposure to high concentrations of oxygen (O_2), the normal rate of cell division in the lung is decreased (1,3). In animals that survive the initial exposure to O_2 or in those exposed to a lower concentration, cell division resumes after about five days (2-4).

Although cell division is inhibited in the lungs of animals exposed to O_2, it is not known whether this is a direct or indirect effect of O_2. Studies *in vitro* have shown that a variety of metabolic processes, including DNA synthesis, are inhibited during exposure to O_2, indicating that O_2 can act directly on the cell. However, in the intact animal, other factors such as stress hormones and starvation also decrease cell division. In animals exposed to O_2, it has been noted that they eat less and lose weight. We (5) have shown that air-breathing mice deprived of food had weight loss and depression in DNA synthesis similar to that observed in mice exposed to 95 percent O_2. These results indicate that indirect factors are associated with the depression of DNA synthesis observed in mice exposed to high O_2.

Related questions of interest arose: Is the type 2 cell proliferation induced by injury of the lung inhibited by high O_2, and is decreased food consumption a factor? Our data (see manuscript #6 in the detailed part of this report) indicate that lack of food or 40 percent O_2 did not inhibit type 2 division associated with repair of ozone damaged alveolar epithelium. Oxygen concentrations of 61 percent or higher did inhibit reparative cell division in ozone damaged alveoli of mice.

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B. DETAILS OF RESULTS

Preface to Section B

This section is structured so that copies of the publications that resulted from the ONR support are used. In one instance a draft manuscript is included. Advantages of this structure are that considerable detail is included in a cost-effective way and contributors to the various studies are listed as authors or otherwise acknowledged. A disadvantage is inconsistency in format.

1

CELL PROLIFERATION IN LUNGS OF MICE EXPOSED TO ELEVATED CONCENTRATIONS OF OXYGEN

Cell Proliferation in Lungs of Mice Exposed to Elevated Concentrations of Oxygen

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EVANS, M. J., and J. D. HACKNEY. *Cell proliferation in lungs of mice exposed to elevated concentrations of oxygen.* Aerospace Med. 43(6):620-622, 1972.

The purpose of this study was to determine the effects of concentrations of O_2 greater than 21% but less than 100% on cell division in the pulmonary alveoli. To accomplish this mice were exposed to 40, 60, or 80% O_2 and cells preparing to divide were labeled with tritiated thymidine (^{3}H-TdR). Labeled cells were visualized with autoradiographic techniques and labeled cells counted with the light microscope and expressed as a labeling index.[5]

In the present study it was shown that DNA synthesis in endothelial cells also was inhibited by exposure to less than 100% oxygen. However, the other cell types were not affected. Inhibition of DNA synthesis lasted about five days and then returned to control levels or above. Since the animals were continuously exposed to oxygen, these results indicated that the cells had adapted to the oxygen environment. Presumably, before they adapted to the oxygen, processes associated with cell division (such as tissue repair and cell turnover) would be inhibited.

IN PREVIOUS REPORTS it was shown that 100% oxygen inhibits cell division in pulmonary alveolar cells of the intact mouse.[6,8] All cell types were inhibited after three days of exposure and all of the mice died after five days of exposure to 100% oxygen. In studies using lesser concentrations of oxygen and different species of animals, there appeared to be proliferation of cells if they lived longer than one week.[13,17]

The purpose of this study was to determine the effects of concentrations of O_2 which are less than 100% on cell division in the pulmonary alveoli. To accomplish this, mice were exposed to 40, 60, or 80% O_2 and cells preparing to divide were labeled with tritiated thymidine (^{3}H-TdR). Labeled cells were visualized with autoradiographic techniques, counted with the light microscope, and expressed as a labeling index.[5]

MATERIALS AND METHODS

Young male mice (Swiss-Webster) were used in this study. The mice were exposed to oxygen by placing them in a closed chamber containing CO_2 absorbent (Sodasorb) with either 40, 60, or 80% oxygen flowing at a rate of 2½ L/min. The oxygen was humidified by bubbling through distilled water and the chamber temperature was maintained at 23°C. ±2.

One hour before sacrifice the mice were removed from the chamber, injected intraperitoneally with 50 microcuries of ^{3}H-TdR (S.A. 7.2 Ci/mM) and returned to the chamber. The mice were killed with an overdose of sodium pentobarbital, the lungs removed and fixed by perfusion with 2% gluteraldehyde, sliced, and post-fixed in 1% OsO_4. Pieces of the tissue, each containing a terminal bronchiole, were embedded flat in Beem capsules with Araldite. One-micron sections for light microscopic autoradiography were cut and coated with Ilford L-4 emulsion. Following exposure in the dark, the slides were developed, fixed, and stained with toluidine blue. Details of the procedures for tissue preparation and autoradiography have been presented previously.[10]

Cells that had incorporated ^{3}H-TdR into their nuclei (labeled cells) were counted and expressed as a proportion of the total cell population. Approximately 2,000 labeled and unlabeled cells were counted in 4 different microscope sections from each animal. The labeled cells were then divided into 3 groups: (a) Type 2 alveolar cells (b) alveolar macrophages (c) cells in the alveolar walls.

Each group of cells was then expressed as labeled cells per 1000 cells counted. Red blood cells were not included in the count. When changes from control values were noted in the group of labeled cells from the alveolar wall, the labeled endothelial cells were identified and their relative increase or decrease determined.

Due to the small number of experimental animals involved, all data points are reported so that trends can be observed; statistical analysis did not seem warranted.

RESULTS

General Observations—A total of 90 mice were used in these experiments. Twenty-four served as controls, 21 were exposed to 40% oxygen, 23 exposed to 60% oxygen, and 22 exposed to 80% oxygen. None of the animals died during the experiment. No evidence of edema or hyaline membranes was observed in the lungs of control mice or those exposed to either 40, 60, or 80% oxygen.

This research was supported by the office of Naval Research, N00014-70-C-0306 and NHLI contract No. NIH-71-2151.

Labeling Indexes–The control groups of 24 mice had a mean labeling index of 1.5 Type 2 alveolar cells/1,000 alveolar cells ±1.3, 1.2 alveolar macrophages/1,000 aveolar cells ±1.4, and 20.5 cells in the alveolar walls/1000 alveolar cells ±9.6. This last group of labeled cells is composed mainly of agranular leukocytes and endothelial cells. Examples of labeled cells and the proportion each makes of the total labeled cell population in the alveoli have been presented previously.[7,8,10]

The group of mice exposed continuously to 40% oxygen for eight days had no change in the number of Type 2 alveolar cells or alveolar macrophages that could be labeled with ^{3}H-TdR. Labeling indices for Type 2 alveolar cells ranged from 0.4 to 3.0 labeled cells per 1,000 alveolar cells, and alveolar macrophages from 0 to 4.5 labeled cells per 1,000 alveolar cells. Figure 1 illustrates the labeling indices for the remaining labeled cells in the alveolar walls. Initially, the labeling indices were slightly depressed, but after four days they had returned to control levels and above. The increased labeling indices are due mainly to more labeled endothelial cells and agranular leukocytes.

The group exposed continuously to 60% oxygen for nine days had no change in the number of Type 2 alveolar cells or alveolar macrophages that could be labeled. Labeling indices for Type 2 alveolar cells ranged from 0.5 to 2.2 labeled cells per 1,000 alveolar cells and alveolar macrophages from 0 to 3.2 labeled cells per 1,000 alveolar cells. Figure 2 illustrates the labeling indices for the remaining cells in the alveolar walls. Initially, the labeling indices were depressed but by five days they had returned to control levels. The initial decrease in the labeling index was due to fewer labeled endothelial cells.

In the group of mice exposed continuously to 80% oxygen there were no changes in the number of labeled Type 2 alveolar cells or alveolar macrophages. The labeling indices for Type 2 alveolar cells ranged from 0 to 2.0 labeled cells per 1,000 alveolar cells and alveolar macrophages from 0.5 to 2.9. Figure 3 illustrates the labeling indices for the remaining cells in the alveolar walls. Again there was an initial decrease in the number of labeled cells which returned to control levels by six days. The decreases were due to fewer labeled endothelial cells.

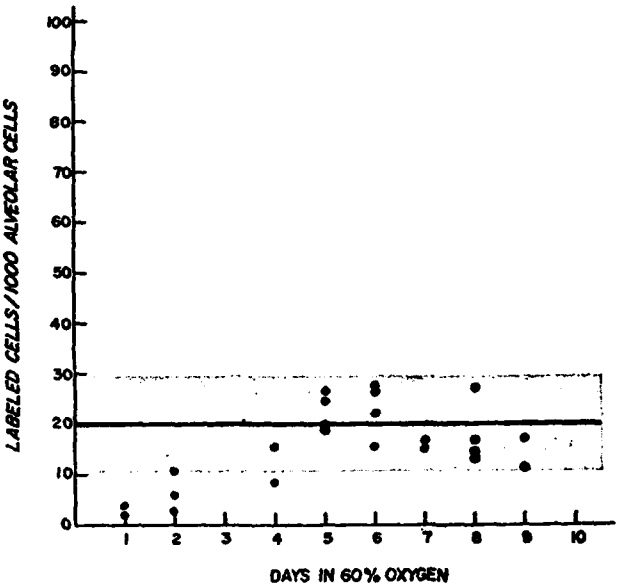

Fig. 2. Labeling index of cells in the alveolar walls during exposure to 60% oxygen. The solid line represents the mean and the shaded area the standard deviation of the mean of control animals.

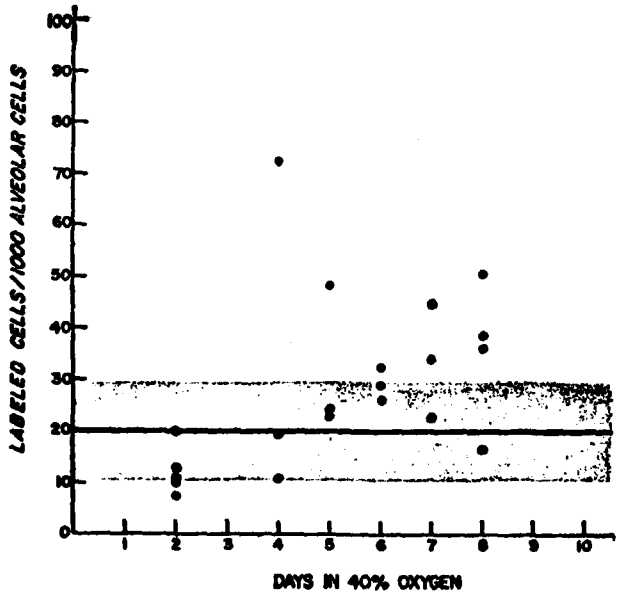

Fig. 1. Labeling index of cells in the alveolar walls during exposure to 40% oxygen. The solid lines represents the mean and the shaded area the standard deviation of the mean of control animals.

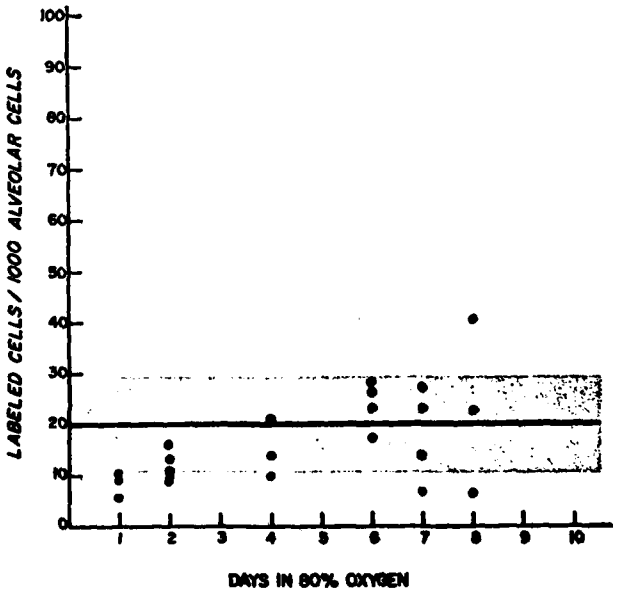

Fig. 3. Labeling index of cells in the alveolar walls during exposure to 80% oxygen. The solid line represents the mean and the shaded area the standard deviation of the mean of control animals.

DISCUSSION

It is well established that high concentrations of oxygen inhibit growth of cells *in vitro*[12]; DNA synthesis is inhibited and the ability of the sister cells to separate is impaired.[11] However, less is known about the effect of oxygen on cell division *in vivo*. Evans et al.[8] showed that 100% oxygen inhibits DNA synthesis in cells of the pulmonary alveoli of the intact mouse. Alveolar macrophages were inhibited by 72 hours of exposure to O_2, Type 2 alveolar cells after 48 hours, and endothelial cells were affected by 24 hours. In the present study it was shown that DNA synthesis in endothelial cells was also inhibited with lesser concentrations of oxygen. However, the other cell types were not affected. The reason for the different responses of the pulmonary cell types is not clear, but it is thought to be due to intracellular concentrations of metal ions, chelating agents, or sulfhydral groups.[12] In addition, *in vitro* studies have shown that the degree of inhibition is dependent on the concentration of oxygen used.[2, 11, 16] This would mean that the most sensitive cell types would be the ones most likely to be affected by a lesser concentration of oxygen. This agrees with the present finding that endothelial cells, the most sensitive to 100 percent oxygen, were the only ones affected in this study.

Inhibition of DNA synthesis lasted about five days and then returned to control levels. Since the animals were continuously exposed to oxygen, these results indicate that the cells had adapted to the oxygen. However, before they adapted to the oxygen, processes associated with cell division, such as tissue repair and cell turnover, were inhibited.

In the present study there was no visible tissue damage so inhibition of repair would not affect the animal. However, in animals exposed to higher levels of oxygen inhibition of repair processes is important. The initial stages of the oxygen lesion are focal damage to endothelial cells, formation of hyaline membranes, edema, and loss of Type 1 alveolar epithelium.[4, 14] In animals that survive the initial damage repair of the tissue begins after about seven days.[13] At this time there is proliferation of endothelial cells in capillaries and Type 2 alveolar cells in the epithelium in order to repair damaged areas of tissue.[13, 15] The beginning of cell proliferation around seven days reported by Kapanci et al.[13] compares favorably with the present results, which showed that cell division was inhibited for only the first five days and had returned to control levels by seven days. Although Type 2 cells were not inhibited in this study, they were after 48 hours when exposed to 100% oxygen.[8] In the previous study it was not possible to determine whether the Type 2 cells adapted to the O_2 and began dividing again because the animals died by five days.[8] From the work of others it can be assumed that animals which survive more than five days adapt and begin to repair the tissue. This indicates that one of the factors in oxygen toxicity may be the inability to repair tissues damaged during the early days of exposure to oxygen because the cells are inhibited from dividing. This can be compared with the NO_2 lesion in which there is extensive tissue damage but no inhibition of cell division. In this case repair begins almost immediately and there are very few animal deaths.[10]

Cells of the pulmonary alveoli also constitute a renewing cell population.[1, 7] In renewing cell populations there is cell division but no net increase in the number of cells because one sister cell is sloughed off.[5] The purpose of a renewing cell population is to maintain the integrity of a tissue by acting as a source of new cells, e.g., bone marrow and intestine. How this relates to oxygen toxicity in the lung is not clear. Because cells are inhibited by oxygen it may be implied that the integrity of pulmonary tissue is threatened. However, further research is necessary concerning the role of cell renewal in pulmonary alveoli before the effects of O_2 on this system can be completely understood.

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2

ANIMAL ATMOSPHERIC EXPOSURE CHAMBER SYSTEM USING A MODIFIED TANK RESPIRATOR

Animal Atmospheric Exposure Chamber System Using a Modified Tank RESPIRATOR

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A low-cost chamber for exposing small animals to artificial atmospheres is described. Two surplus tank respirators for humans were modified for use, one as an experimental chamber and the other as a control chamber. They were designed so that high flows of gas would maintain O_2, CO_2, water vapor, and waste-generated gases at acceptable levels on a once-through basis. Procedures for waste removal, feeding and watering are also described. The changes in environmental chamber conditions during exposure of squirrel monkeys to 100% O_2 and mice to 40% O_2 are reported. This simple, inexpensive system is adequate for experimental exposure of up to 13 squirrel monkeys where only limited control of the environment is required. Excellent environmental control was achieved with up to 30 mice.

A SERIES OF STUDIES on the effects of altered O_2 concentrations on turnover times of alveolar cells and lung morphological changes are being conducted in our laboratory using squirrel monkeys. A review of the literature on small animal chambers revealed (1) relatively expensive environmental chambers for long-term small animal exposures have been built,[1-4] (2) inexpensive, fabricated exposure systems were limited as to the size and number of animals that could be accommodated,[5,6] and (3) large, complex chambers with sophisticated control systems that were already in existence have been used.[7-11] For a variety of reasons, none of these chamber designs were appropriate for our specific application. Our goal was to provide a chamber that was simple in design, minimal in cost, and yet large enough to handle up to 13 squirrel monkeys for several days.

Chamber Description

A tank respirator of the "iron lung" variety appeared to be suitable for housing a number of squirrel monkeys for several days. These devices are rarely used today in this hospital, and, consequently, there was a large assortment in surplus storage. Two 36″ by 72″ respirators served as the experimental chamber and the control chamber.

The tanks were modified to meet the requirements of an animal exposure chamber. The bellows and motors were removed and all porthole collar gaskets were replaced with solid discs of the same material to reduce leakage. The 18″ by 8″ bedpan port was left intact to provide access to the inside of the chamber. One-inch holes for gas in-flow and out-flow tubing were drilled at opposite ends of the tanks, and in the bottom of both tanks for the drainage of liquid material.

Perforated stainless steel sheeting to serve as a floor for the chamber was installed on the sliding framework attached to the tank door. A 6″ wide raised platform where the animals could perch was installed along the length of the floor. (Figure 1 shows the overall chamber configuration and later modification described in the next section.)

A hole for the insertion of the metal tubing spout of a standard animal watering bottle was drilled in the middle of the cham-

This research was supported by the office of Naval Research N00014-70-C-0306 and NHLI NIH-71-2151.

ber between the Plexiglass windows. The bottle itself was mounted with a bracket and tension spring arrangement on the outside of the tank. The monkeys were fed by placing stainless steel bowls of fruit and dry food directly inside the chamber.

Temperature and humidity were monitored inside the chambers by visual inspection of a Weather Measure TM50 dial thermometer and an HM15 dial Durotherm Hygrometer, respectively. Brackets mounted the gauges so they were clearly visible through one of the Plexiglass windows.

A manifold of four O_2 cylinders supplied O_2 to the O_2 chamber metered through a wall-mounted NCG O_2 flow meter. O_2 concentration was measured periodically at the out-flow with a Beckman E2 O_2 analyzer and the CO_2 concentration was measured with with a Beckman LB-1 Medical Gas Analyzer.

Operational Procedures and Results

Exposure of Squirrel Monkeys to 100% O_2

For an initial three-day 100% O_2 exposure experiment, a once-through O_2 flow was used. Sodasorb absorbent pellets in three 18" by 12" aluminum foil baking pans were placed under the perforated steel flooring at the bottom of each chamber.

Although O_2 and CO_2 concentrations and temperatures were adequately controlled (see Table I), relative humidity rose to 100%. Presumably, the presence of urine and feces in the bottom of the chamber and moisture from fruit slices fed to the animals contributed to the high humidities.

In an attempt to reduce these humidity levels in the O_2 chamber, the bank of four O_2 cylinders was supplemented by an additional four-cylinder manifold meter by a second NCG O_2 flow meter. A second O_2 inlet hole was drilled in the chamber wall directly opposite the first. A high-flow capacity Dynapump (25 lpm) was connected in parallel with the original pump (8 lpm) for higher flow rates into the control chamber. Since the Sodasorb was wet and probably deactivated during the 72-hour study, it was eliminated. The high flow rates

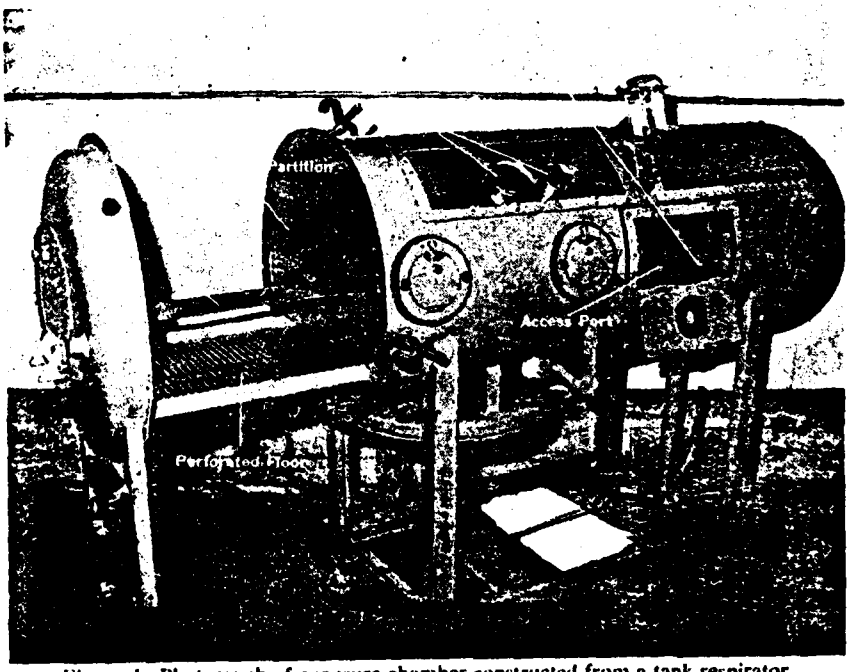

Figure 1. Photograph of exposure chamber constructed from a tank respirator.

TABLE I
Summary of Chamber Parameters
during a 72-Hour and a 120-Hour Exposure of Squirrel Monkeys

Parameter	72-Hour Exposure		120-Hour Exposure	
	O_2 Chamber	Control Chamber	O_2 Chamber	Control Chamber
Gas inflow, lpm	4–10	4—10	*14–30	33
O_2 concentration, %	99.3–100 (Dry)	Room Air	99–100	Room Air
Max. CO_2 concentration, %	1.4	0.5	<1.0	<1.0
Chamber Temperature °F.	78–85	72–81	82–84	81–83
Chamber % of relative humidity	59–100	61–100	61–90	68–93
Initial No. of animals	13	6	13	6
Room temperature °F.	72–83		80–82	

NOTE: Double values represent range of parameter variation.
*Initial O_2 flow was 30 lpm but reduced to a final flow of 14 lpm.

through the chambers would, presumably, maintain CO_2 at a low level.

Additional modifications were made in both tanks to reduce the water vapor content. An adidtional one-inch hole was drilled in the bottom of the tank at the door flange and fitted with a stopper. The tanks were tipped slightly toward this end using the elevation mechanism at the other end of the tank. This allowed the urine to collect and it could then be drained by removing the stopper. Fecal matter deposited on the perforated flooring and on the bottom of the tank was periodically removed.

A two-foot diameter steel partition was attached to the end of the sliding framework so that, when the door was unlatched and the entire framework rolled out of the chamber, the partition served to keep the animals on the flooring and out of the bottom of the tank. Another removable partition of the same diameter but with cutouts for the raised platform and the area beneath the flooring was positioned to cover the upper part of the open end of the chamber when the door was first pulled away from the chamber. This double-partition permitted the framework to be pulled out, but restrained the monkeys between the two partitions. Feces and pooled urine in the bottom of the tank could then be scraped toward the front and removed using a metal scraper blade mounted on a handle. (Figure 1 shows the double partition arrangement.)

To further reduce moisture, orange and apple slices were placed in the chamber for a limited time. Peels and leftovers were removed as soon as the animals finished eating.

The chambers were located in a room with thermostatically controlled temperature and a heat pump was used for cooling or heating. The internal chamber temperatures could be adequately controlled by varying the room temperature. Table I summarizes the chamber parameter ranges for both the 72-hour and the 120-hour experiments.

Exposure of Mice at 40% O_2

This system has also been used for O_2 exposure experiments with up to 30 mice contained in separate cages within one tank. A Veriflow MR-1 ventilator O_2 controller was used to dilute 100% O_2 with cylinder compressed air to 40% O_2. The flow rate was set at 13 liters per minute. This experiment lasted for 20 days and, under these conditions, the temperature remained very stable and the relative humidity remained near 50%.

Discussion

The once-through flow technique was used primarily to eliminate the need for an environmental control system and thus minimize

cost of fabrication and operation of the chamber. In adidtion to economy, certain other advantages are inherent in this kind of system. Leakage is not an important consideration since gas outflow from the chamber is much greater than even a sizable leak.

A certain flexibility exists with a once-through operation since oxygen flow rate can be readily adjusted to maintain O_2 concentration and control CO_2 concentration, temperature and humidity. Changes in these parameters occur when the number of animals in the chamber is altered, following contamination of the chamber atmosphere by removal of the animals, or after cleaning the chamber. The simple design of the system reduces the possibility of component failures, which are more likely in a complex environmental control loop.

Certain disadvantages exist in this type of design, however. Large quantities of O_2 are used, thus necessitating frequent cylinder changes. Careful planning and coordination are required to assure an adequate supply of cylinders for a particular test duration. Frequent monitoring of the O_2 flow rate and cylinder gas supply is required to maintain the chamber atmosphere.

Controlling humidity is the most significant problem, however. Water vapor from urine and fresh fruit contributes to the creation of high humidities.

For tests of longer duration, it appears that some kind of automatic environmental control system would be worth considering to minimize the amount of O_2 required and to control humidity. For tests lasting a few days, the flow-though system is entirely adequate for animals such as squirrel monkeys that tolerate high humidities. The system is also adequate for exposures of longer duration using small animals, such as mice, which produce smaller amounts of waste, CO_2 and water vapor.

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3

EFFECTS OF 100% OXYGEN ON CELL DIVISION IN LUNG ALVEOLI OF SQUIRREL MONKEYS

Effects of 100% Oxygen on Cell Division in Lung Alveoli of Squirrel Monkeys

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HACKNEY, J. D., M. J. EVANS, and C. E. SPIER. *Effects of 100% oxygen on cell division in lung alveoli of squirrel monkeys.* Aviat. Space Environ. Med. 46(11):1340-1342, 1975.

The purpose of this study was to determine the effects of 100% oxygen on cell division in lung alveoli of squirrel monkeys. To accomplish this, squirrel monkeys were exposed to 100% oxygen for up to 5 d. Prior to sacrifice, cells preparing to divide were labeled with tritiated thymidine (^{3}H-Tdr). Labeled cells were visualized with autoradiographic techniques, counted with the light microscope, and expressed in terms of a labeling index. In the present study it was shown that DNA synthesis was initially inhibited by exposure to 100% oxygen. However, within 3 d it was returning to normal and by 5 d was well above control levels. Analysis of the cell types involved showed that the large increase in labeling was due to an increase in dividing Type 2 cells, which is thought to be for replacement of damaged Type 1 cells.

THE RESPONSE of alveolar tissue to elevated concentrations of oxygen has been studied for many years. Morphologically, these studies have shown that during the first 2 d of exposure there is little evidence of tissue damage. After this time, subtle changes occur in endothelial cells and more dramatic ones in the Type 1 epithelium. Type 1 cells appear to become damaged, slough off, and be replaced by a cuboidal epithelium of Type 2 cells (2,7,9). During recovery, the Type 2 cells may transform into Type 1 cells as the tissue returns to normal (1,6). At the cellular level, there appears to be a general depression of the metabolic activity, initially through inhibition of enzymes and biochemical processes. However, later, as tolerance develops, metabolic and various enzymatic activities increase (8). This sequence of events represents tissue damage, repair, and adaptation of the tissue to a hyperoxic environment.

The purpose of the present study was to determine the proliferative response of the pulmonary alveoli in monkeys to continuous exposure to 100% oxygen. In previous studies with mice, there was a decrease in the ability of lung cells to divide during exposure to oxygen (3). However, in animals that survived the early phase of exposure, the number of dividing cells returned to control levels or above (4). In the present study, monkeys were exposed to 100% oxygen for 5 d. At intervals, the animals were removed from the chambers, injected with tritiated thymidine (^{3}H-TdR) in order to label dividing cells, and the lungs removed and prepared for light microscopic autoradiography. The number of dividing cells in the alveoli was then determined by examining autoradiographs with the light microscope.

MATERIALS AND METHODS

Thirty-six male squirrel monkeys *(Samimiri sciureus)* were used in this study. Experimental animals were exposed to 98-99% oxygen at ambient pressure for 1, 2, 3, 4, and 5 d in two human tank respirators modified for use, one for an experimental chamber and the other for a control chamber (10). They were designed so that high flows of gas (oxygen mixtures for the experimental chamber and compressed room air for the control chamber) on a once-through basis would maintain oxygen, carbon dioxide, water vapor, and waste-generated gases at acceptable levels. Flows in both chambers were sufficient to keep the CO_2 levels less than 1%. This was confirmed by gas chromatographic analysis. Mean exposure chamber temperatures for the individual runs ranged from 80°-83°F and mean relative humidity values ranged from 88-95%. In all, 25 animals were exposed. Eleven additional control animals were maintained under similar conditions but breathed ambient air. Mean control chamber temperatures ranged from 77°-83°F and mean relative humidity values ranged from 87-89%. One hour before sacrifice, the animals were given an intraperitoneal injection of tritiated thymidine (specific activity 6.7 Ci/mmol 3.3×10^{-3} mCi/g body weight), in order to label cells preparing to divide. The animals were sacrificed with an overdose of sodium pentobarbital and the intact lungs removed from the animals. The trachea was then cannulated and 2% glutaraldehyde in cacodylate buffer perfused into the airways under 12 cm of water pressure. After fixation for 1 h, portions from the upper, middle, and lower regions of the left lobe were sliced, washed in a buffer, and postfixed in 1% osmium tetroxide buffered at pH 7.4 with sodium cacodylate plus 7.5% sucrose. Following dehydration and infiltration with araldite, portions of the lung containing terminal and respiratory bronchioles and the adjacent alveoli were dissected out and embedded flat in Beem capsules. One-micron sections were cut from two capsules from each region and prepared for light autoradi-

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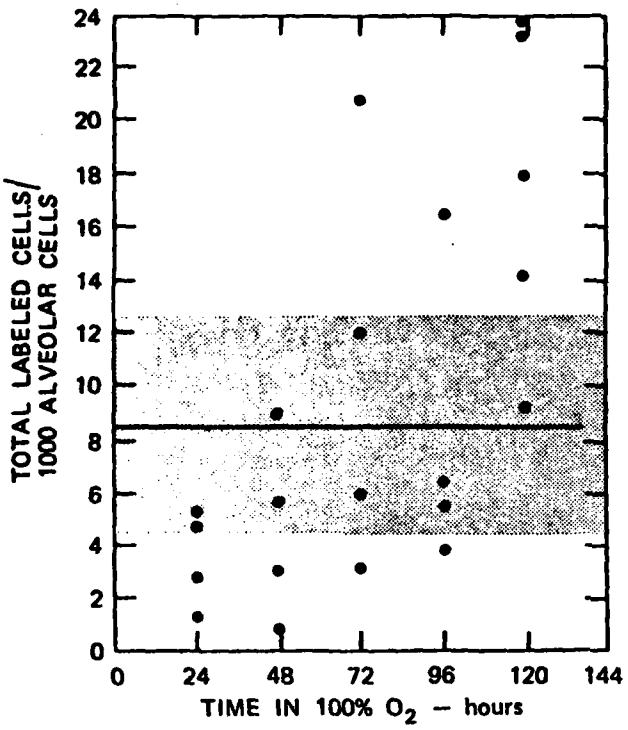

Fig. 1. Total labeled cells per 1000 alveolar cells during exposure to 100% oxygen. The solid line represents the mean and the shaded area the standard deviation of the mean of control animals.

ography (6).

After 6 weeks' exposure, the autoradiographs were developed and stained with toulidine blue. The sections were studied under the light microscope and approximately 3000 cells were counted from three different regions of the lung. The number of labeled Type 2 cells, alveolar macrophages and cells in the alveolar wall—this last group made up mainly of leucocytes in the capillaries and endothelial cells—were determined. The labeling index was presented as labeled cells (of a particular type) per 1000 alveolar cells counted (5,6).

RESULTS

General Observations: Mortality was high in the experimental animals, with two dead at 96 h and two more by 120 h.* Histologically, a progressive perivascular edema was found plus some tissue destruction in the sacrificed experimental animals. Details of the histological changes will be presented in another paper.

Labeling Indexes: Labeled cells in control and experimental animals appeared randomly distributed throughout the tissue. Eleven control animals had an overall mean labeling index (LI) of 8.1 ± 4.0 labeled cells per 1000 alveolar cells. Expressed as cell types, the LI of Type 2 cells was 0.4 ± 0.3, alveolar macrophages 0.3 ± 0.3, and cells in the alveolar wall (CAW) 7.4 ± 3.4. The total number of labeled cells found in exposed, compared to control, lung tissue indicates there was an initial decrease followed by an increase during continuous 100% oxygen inhalation (Fig. 1). When broken down according to cell type, it was found that the increase was due mainly to more labeled Type 2 cells in alveoli. Few changes were seen in the alveolar macrophage population. The LI of CAW at 24 h was below control levels with a mean of 2.3 (Table I). The low LI was due primarily to fewer labeled endothelial cells in the capillaries. These labeling indexes gradually increased and by 5 d had returned to control levels. At this time, labeled leucocyte and endothelial cells were observed (Table I).

The labeling indexes of Type 2 cells initially were the same as controls with an LI of 0.4 (Table I). This figure increased and by 5 d was well above the control LI of 0.4, with a mean of 7.6. The increased labeled cells were in groups scattered throughout the alveoli. They did not appear to be associated with alveoli near the openings of the terminal or respiratory bronchioles as was observed during exposure to nitrogen dioxide.

*These animals were submitted to necropsy; in each case, advanced autolysis precluded histopathology.

EXPLANATION OF ABBREVIATIONS

CAW	— cells in alveolar wall
F	— fahrenheit
^{3}H-Tdr	— tritium-labeled thymidine
LI	— labeling index (tritium-labeled cells per 1000 cells counted)
NO_2	— nitrogen dioxide
PPM	— parts per million (by volume)

DISCUSSION

The results of this study show an initial decrease in the number of dividing cells in the pulmonary alveoli of monkeys exposed to 100% oxygen. This is followed by a return to control levels or above for cells in the alveolar walls and an increase above controls for Type 2 cells. In a previous report covering mice exposed to 100% oxygen, there was a large decrease in the number of labeled cells in the alveoli (3). In that experiment no animals lived longer than 4 d. The initial depression in cell labeling reported was confirmed in an article by Bowden *et al.* concerning mice exposed to 100% oxygen (2). They found a maximum decrease after 4 d of exposure. In another study, using lower concentrations of oxygen (40, 60, and 80%), there also appeared to be an initial decrease in cell labeling, which returned to control levels by 7 d (4). In these experiments, most of the animals survived the exposure. Similar results reported from studies of microorganisms and cells in culture suggest initial depression in cell division may also be a general phenomenon in pulmonary tissue during the early stages of exposure to high concentrations of oxygen (8).

The increase in cell labeling at 4-5 d in the present study was also demonstrated in a previous study, using lower concentrations of oxygen (4), and in several papers by other investigators concerning the pathogenesis of oxygen toxicity (2,3,8). Those papers re-

TABLE I. LABELING INDEX OF ALVEOLAR TISSUE DURING EXPOSURE TO 100% OXYGEN.

	LABELED CELLS PER 1,000 ALVEOLAR CELLS					
DAYS	Type 2 Cells	p**	Alveolar Macrophages	p**	Cells in the Alveolar Walls	p**
1	0		0		0	
	1.1		1.1		2.9	
	0.6		0		2.1	
	0	N.S.	0.7	N.S.	4.2	<0.02[1]
2	0		0		5.7	
	4.1		0		4.9	
	0		0		0.7	
	0	N.S.	0	N.S.	3.2	N.S.
3	2.4		0		9.6	
	1.1		0.4		4.4	
	0		0		3.2	
	11.7	N.S.	0	N.S.	9.2	N.S.
4	1.3		0		4.0	
	1.3		0.7		3.4	
	1.1		0		2.8	
	5.9	<0.02	0	N.S.	7.4	N.S.
5	6.4		0		7.8	
	14.5		1.1		8.4	
	4.0		0.7		13.2	
	3.7		1.2		4.3	
	9.5	<0.002	1.2	<0.02	13.0	N.S.
Controls* (m ± 1S.D.)	0.4 ± 0.3		0.3 ± 0.3		7.4 ± 3.4	

* N = 11

**p is for each experimental day vs. control; Mann-Whitney U (nonparametric) test was used.

[1] experimental group less than control.

ported hyperplasia of alveolar cells in animals exposed to oxygen for more than 5 d. The principal multiplying cells were Type 2 cells and endothelial cells. The purpose of their multiplication is believed to be repair of damaged tissues. This was recently confirmed by Adamson and Bowden in mice during recovery from exposure to oxygen (1). The concept of tissue repair during continuous exposure to high concentrations of oxygen is well illustrated in the paper by Kapanci, *et al.* (9). In that study, they found evidence of Type 1 cell damage in the lungs of monkeys after 3 d of exposure to oxygen. The damaged cells then appeared to be sloughed off. After 7 d of continuous exposure, the damaged areas appeared to be replaced by a cuboidal epithelium composed of Type 2 cells. Additional papers (2,7) that studied different species have confirmed the morphological findings of Kapanci, *et al.* (9).

The present study illustrates that multiplication of Type 2 cells begins after 4-5 d of exposure. Presumably, this activity would result in the hyperplasia after 7d reported by Kapanci, *et al.* (9). An additional factor associated with increased Type 2 cell division may be the development of tolerance in the animal. In an article by Evans, *et al.* using rats exposed continuously to 15 ppm NO_2, it was found that when Type 1 cells were damaged, Type 2 cell proliferation occurred, reached a peak at about 3 d, and then returned to normal despite continuous exposure (5). This demonstrates that the tissues developed some tolerance to the NO_2.

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4

EFFECTS OF 60 AND 80% OXYGEN ON CELL DIVISION IN LUNG ALVEOLI OF SQUIRREL MONKEYS

Effects of 60 and 80% Oxygen on Cell Division in Lung Alveoli of Squirrel Monkeys

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HACKNEY, J. D., M. J. EVANS, and B. R. CHRISTIE. *Effects of 60 and 80% oxygen on cell division in lung alveoli of squirrel monkeys.* Aviat. Space Environ. Med. 46(6):791-794, 1975.

The purpose of this study was to determine the effects on cell division of 60 and 80% oxygen in the lung alveoli of squirrel monkeys. To accomplish this, squirrel monkeys were exposed to 60 and 80% oxygen for up to 8 d. Prior to sacrifice, cells preparing to divide were labeled with tritiated thymidine (^{3}H-TdR). Labeled cells were visualized with autoradiographic techniques, counted with the light microscope, and expressed in terms of a labeling index. In the present study, it was shown that DNA synthesis was not changed by exposure to 60%; however, with exposure to 80% oxygen, cell labeling was well above control levels by 5 d. Analysis of the cell types involved showed an increase in labeling due to an increase in dividing Type 2 cells. Other cells in the alveolar wall also showed an increase in labeling.

MORPHOLOGICAL studies of alveolar tissues exposed to concentrations of oxygen (O_2) near 100% have shown that during the first 2 d there is little evidence of tissue damage. After this time, subtle changes occur in endothelial cells and more dramatic ones in the Type 1 epithelium. Type 1 cells appear to become damaged, slough off, and are replaced by a cuboidal epithelium of Type 2 cells (2,8,11). During recovery, the Type 2 cells may transform into Type 1 cells as the tissue returns to normal (1,7). At the cellular level, there appears to be a general depression of the metabolic activity, initially through inhibition of enzymes and biochemical processes. Later, as tolerance develops, metabolic and various enzymatic activities increase (10). This sequence of events apparently represents tissue damage, repair, and adaptation of the tissue to a hyperoxic environment. In another study, using squirrel monkeys (9), it was shown that cell labeling was initially inhibited by exposure to 100% O_2. However, within 3 d it was returning to normal and by 5 d was well above control levels. Analysis of the cell types involved showed that the large increase in labeling was due to an increase in dividing Type 2 cells.

This research was supported by the Office of Naval Research, contract No. N00014-70-C-0306 and NHLI grant No. HL-15098 and contract No. NIH-71-2151.

The purpose of the present study was to determine the response of the pulmonary alveoli in squirrel monkeys to continuous exposure to 60 and 80% O_2. In previous studies with mice, there was a decrease in the ability of lung cells to divide during exposure to 60 and 80% O_2 (4). However, in animals that survived the early phase of exposure, the number of dividing cells returned to control levels or above (5). In the present study, monkeys were exposed to 60 and 80% O_2 for up to 8 d. At intervals, the animals were removed from the exposure chambers, injected with tritiated thymidine (^{3}H-TdR) in order to label dividing cells, and the lungs removed and prepared for light microscope autoradiography. The number of dividing cells in the alveoli was then determined by examining autoradiographs with the light microscope.

MATERIALS AND METHODS

60% O_2 *Exposure*: 16 male squirrel monkeys (*Saimiri sciureus*) were exposed to 60% O_2 at ambient pressure—for 2, 4, and 8 d for experimentals and 2 and 8 d for controls—in two human tank respirators modified for use, one as an experimental chamber and the other as a control chamber (12). They were designed so that high flows of gas (oxygen mixtures for the experimental chamber and compressed room air for the control chamber) on a once-through basis would maintain oxygen, carbon dioxide, water vapor and waste-generated gases at acceptable levels. Flows in both chambers were sufficient to keep the CO_2 levels less than 1%. This was confirmed by gas chromatographic analysis. Mean exposure chamber temperature was 78±2°F (25.6±1.2°C) and mean relative humidity was 86±8%. Sixteen animals in all were exposed. Four additional control animals were maintained under similar conditions, but breathed ambient air. Mean control chamber temperature was 73±1°F (22.8±0.6°C) and mean relative humidity was 83±5%. Approximately 1 h before sacrifice, the animals were given an intraperitoneal injection of tritiated thymidine (1 μCi/g body weight) in order to label cells preparing to divide. The animals were sacrificed with an overdose of sodium pentobarbital and the intact lungs removed from the animals. The trachea was then cannulated and

2% glutaraldehyde in cacodylate buffer perfused into the airways under 20 cm of water pressure. After fixation for 1 h, portions from the upper, middle, and lower lobes of the left lung were sliced, washed in a buffer, and postfixed in 1% osmium tetroxide buffered at pH 7.4 with sodium cacodylate plus 7.5% sucrose. Following dehydration and infiltration with araldite, portions of the lung containing terminal and respiratory bronchioles and the adjacent alveoli were dissected out and embedded flat in Beem capsules. One micron sections were cut from two capsules from each region and prepared for light autoradiography (7).

After 6 weeks exposure, the autoradiographs were developed and stained with toluidine blue. The sections were studied under the light microscope and approximately 3,000 cells were counted from three different regions of the lung. The number of labeled Type 2 cells, alveolar macrophages, and cells in the alveolar wall (this last group is made up mainly of leucocytes in the capillaries and endothelial cells) were determined. The labeling index was presented as labeled cells (of a particular type) per 1,000 alveolar cells counted (6,7).

80% O_2 *Exposure*: Twenty-four male squirrel monkeys (*Saimiri sciureus*) were used in this study. Sixteen animals were exposed to 80% O_2 in nitrogen in an enclosed exposure chamber for 8 d. An additional eight animals were placed in a similar chamber but exposed to room air to serve as controls. Each chamber was monitored for temperature and relative humidity and, in addition, the exposure chamber was monitored for oxygen concentration.

The mean exposure temperature was 80±2°F and the relative humidity was 94±6%. The mean control chamber temperature was 77±2°F (25.0±1.6°C) and the relative humidity was 98±2%.

Animals were sacrificed after exposure for 2, 4, 5*, and 8 d. Thirty minutes before sacrifice, the animals were injected intraperitoneally with tritiated thymidine at a dose of 1 μCi/g of body weight. At sacrifice the lungs were fixed, infiltrated, and then embedded flat in Beem capsules. The tissue was processed for autoradiography and histopathological evaluations.

RESULTS

Morphology

60 *and* 80% O_2 *Exposure*: Exposure of squirrel monkeys to 60% O_2 for up to 8 d is associated with minimal and variable morphological changes. Hyperemia and slight edema was observed in lung sections from the 2-d exposed monkeys, slight serofibrinous pleural effusion in 4-d exposed animals, and, in both the exposed and control group monkeys at 8 d, hyperemia and slight edema. These findings were considered inconsequential.

Exposure of squirrel monkeys to 80% O_2 atmosphere from 48 through 192 h is associated with mild generalized lung edema, increased rate of necrobiosis of airway mucosal cells with some mucosal erosion and/or metaplasia, hyperplasia, and cell hypertrophy most apparent in the terminal bronchioles. This experiment was associated with an apparent increase in the number of Type 2 alveolar pneumonocytes in the alveolar walls, and a slight increase in the numbers of alveolar macrophages present in the alveolar lumena.

Labeling Index

60% O_2 *Exposure*: The results of this experiment are presented in Table I. In general, there was no difference between experimental and control animals in the number of labeled Type 2 cells. One animal at 4 d had an elevated labeling index (3.7); however, the others remained within control levels.

There was no difference in the number of labeled alveolar macrophages between experimentals and controls.

Cells in alveolar walls are composed of endothelial cells, agranular leucocytes in the capillaries, and interstitial cells. Endothelial cells and leucocytes together make up the largest proportion of the labeled population. In general, there was no difference between the experimental and control groups except for one animal at 8 d (10.7). In this case, the increased labeling index was due to more agranular leucocytes in the capillaries.

80% O_2 *Exposure*: The results of this experiment are presented in Table II. In general, there was a large increase in the labeling indexes by 8 d. At 2 and 4 d, the mean labeling indexes for Type 2 cells were 1.5 and 1.7. After 5 d this increased to 4.1, and after 8 d to 15.3.

The alveolar macrophages also increased slightly, from 0.5 at 2 d to 2.8 at 8 d.

Cells in the alveolar walls increased from 10.1 at 2 d to 20.0 at 5 d and 57.2 at 8 d. The large increases were due to more labeled agranular leucocytes and endothelial cells. The increased number of labeled cells appeared randomly distributed throughout the alveoli.

DISCUSSION

The results of this study show an increase in cell labeling by 5 d for 80% O_2 but not for 60%. The increase in cell labeling at 4 to 5 d in the present 80% O_2 exposure study was also demonstrated in a previous study using higher concentrations of oxygen (9). The principal multiplying cells were Type 2 cells and endothelial cells. The purpose of their multiplication is believed to be repair of damaged tissues (7). This was recently confirmed by Adamson and Bowden in mice during recovery from exposure to oxygen (1). Several other papers concerning the pathogenesis of oxygen toxicity reported hyperplasia of alveolar cells in animals exposed to oxygen for more than 5 d (2,4,10). The concept of tissue repair during continuous exposure to high concentrations of oxygen is well illustrated in the paper by Kapanci, *et al* (11). In this study they found evidence of Type 1 cell damage in lungs of monkeys after 3 d of exposure to O_2. The damaged cells then

*Animals sacrificed at 5 d were exposed for 4 d, then allowed to breathe ambient air for a 24-h "recovery" period prior to necropsy.

TABLE I. LABELING INDEX OF ALVEOLAR TISSUE DURING EXPOSURE TO 60% O_2.

Time in 60% O_2	Type 2 Cells	Mean	p*	Alveolar Macrophages	Mean	p*	Cells in Alveolar Walls	Mean	p*
2 d	1.0			0.3			5.0		
	0.3	0.5	N.S.	0	0.4	N.S.	2.6	3.2	N.S.
	0.7			1.0			1.8		
	0			0.3			3.4		
4 d	0.6			0			2.4		
	3.7	1.6	N.S.	0.6	0.5	N.S.	4.3	2.9	N.S.
	1.0			0.3			3.1		
	1.0			1.0			1.9		
8 d	0.6			1.3	0.6	N.S.	5.1		
	1.0	0.6	N.S.	0			2.9	5.7	N.S.
	0			1.0			10.7		
	0.6			0.3			4.0		
Controls									
2 d	1.0			0.6			2.1		
	0.3			0			3.0		
		0.8			0.4			2.6	
8 d	0			0.3			2.6		
	1.7			0.6			2.0		

*Each experimental day vs. combined controls; Mann-Whitney U (nonparametric) test was used.

TABLE II. LABELING INDEX OF ALVEOLAR TISSUE DURING EXPOSURE TO 80% O_2.

Time in 80% O_2	Type 2 Cells	Mean	p*	Alveolar Macrophages	Mean	p*	Cells in Alveolar Walls	Mean	p*
2 d**	.7			.7			11.1		
	3.6	1.5	.058	.4	.5	N.S.	8.9	10.1	N.S.
	.9			.6			6.2		
	1.0			.3			14.0		
4 d**	.4			0			10.4		
	3.7			.7			8.0		
	2.5	1.7	N.S.	1.2	.5	N.S.	11.3	8.2	N.S.
	0			.3			3.1		
5 d**	.8			0			10.7		
	6.4			1.7			24.6		
	5.2	4.1	.028	2.9	1.4	N.S.	24.5	20.0	.028
	3.8			1.2			20.2		
8 d***	6.1			3.2			77.5		
	34.0	15.3	.028	4.0	2.8	.058	85.8	57.2	.028
	10.3			1.3			45.5		
	10.6			2.8			20.0		
Controls									
4 d	0			1.5			10.2		
	.3	.3		.3	.5		4.3	5.1	
	0			.3			2.2		
	.7			0			5.7		
8 d	.4			.4			7.4		
	2.2	.7		1.9	.6		11.3	5.7	
	0			0			2.0		
	0			0			2.0		

*Each experimental day vs. control; Mann-Witney U (nonparametric) test was used.
**vs. 4-d control
***vs. 8-d control

appeared to be sloughed off. After 7 d of continuous exposure, the damaged areas appeared to be replaced by a cuboidal epithelium composed of Type 2 cells. Additional papers (2,8) employing different species, have confirmed the morphological findings of Kapanci, *et al* (11).

The present 80% O_2 exposure study illustrates that multiplication of Type 2 cells begins after 4 to 5 d exposure. Presumably, this activity would result in the hyperplasia observed at 8 d. An additional factor associated with increased Type 2 cell division may be the development of tolerance in the animal. In a study by

Evans, *et al.*, using rats exposed continuously to 15 ppm NO_2, it was found that when Type 1 cells were damaged, Type 2 cell proliferation occurred, reaching a peak at about 3 d and then returning to normal despite continuous exposure (6). This demonstrates that the tissues developed some tolerance to the NO_2. It is well known that animals may develop tolerance to oxygen; and the lesions reported during this process are similar to those for animals exposed to NO_2, namely, Type 1 cell damage followed by Type 2 cell hyperplasia. Thus, the same sort of phenomena may occur. Studies to determine the proliferative response during the development of tolerance to O_2 are currently being carried out.**

In the 60% O_2 exposure study, there appeared to be no difference in labeling index due to exposure to 60% O_2 for 8 d. These findings are different from those we reported for mice exposed to 60% O_2 for 10 d (5), in which we showed a depression in the labeling index during the first 2 d of exposure. However, after this time, the labeling index returned to normal and the results were similar.

There is agreement between the histopathology and cell turnover findings for the 80% O_2 exposure experiments in squirrel monkeys.** Agreement between these methods was also reasonable for the 60% O_2 and 100% O_2 exposure experiments in squirrel monkeys.**

Taken together, these findings of little if any effect at 60% and a definite effect at 80 and 100% provide a convincing dose response relationship for O_2 exposure in the squirrel monkey. Comparison with estimates of dose-response effects in man for 60, 80 and 100% O_2, as judged by symptoms and changes in vital capacity (3), suggest that the squirrel monkey can be a useful animal model for predicting effects in man.

**Christie, B. R., J. D. Hackney, and M. J. Evans. Dose response effects of 60, 80 and 100% oxygen on lung structure and cell division in squirrel monkeys. (In preparation)

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5

EFFECTS OF OXYGEN AT HIGH CONCENTRATIONS AND FOOD DEPRIVATION ON CELL DIVISION IN LUNG ALVEOLI OF MICE

EXPERIMENTAL AND MOLECULAR PATHOLOGY 26, 350–358 (1977)

Effects of Oxygen at High Concentrations and Food Deprivation on Cell Division in Lung Alveoli of Mice[1]

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Cell division is inhibited by exposure of mice to high oxygen (O_2). Because the exposed animals consume less food, relative to controls, a question arose about the effect of food deprivation *per se* on cell division. The purpose of this project was to compare the cell division in lung alveoli of animals: breathing air (A), exposed to high O_2 (O), deprived of food (D), and exposed to high O_2 plus deprived of food (OD). Swiss–Webster mice were divided into four equal groups: A, O, D, and OD. Animals from each group were sacrificed daily from 0–5 days. Cells synthesizing DNA and preparing to divide were labeled with tritiated thymidine. Labeled cells were visualized with autoradiography, counted with the light microscope, and expressed as a labeling index. Groups O and D showed statistically similar progressive decreases in cell labeling. Regression analysis testing for differences in trends showed that the combined data from groups O and D differed significantly from both the group A cell labeling, which did not decrease over 5 days, and the group OD cell labeling, which decreased dramatically. Generally similar results were found for body weight changes in the respective groups. These results show that: (1) Complete food deprivation with weight loss causes a decrease in the number of cells synthesizing DNA; (2) exposure to high O_2 causes a decrease in DNA synthesis, similar to that seen with food-deprivation weight loss; (3) exposure to high O_2 results in a decreased food intake and produces about the same weight loss as complete food deprivation; and (4) complete food deprivation of high-O_2-exposed mice produces more weight loss and decreases DNA synthesis even more. Thus, to the extent that it occurs, the decreased-food-associated weight loss during exposure to high O_2 is a principal factor in decreasing DNA synthesis.

INTRODUCTION

During the first day of exposure to high concentrations of oxygen (O_2), the normal rate of cell division in the lung is decreased (Evans *et al.*, 1969). In animals that survive the initial exposure to O_2 or in those exposed to a lower concentration, cell division resumes after about 5 days (Evans *et al.*, 1972; Northway *et al.*, 1972; Adamson and Bowden, 1974; Hackney *et al.*, 1975). The relationship

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of early depression in cell division to the pathogenesis of O_2 toxicity may occur through retardation of the normal repair processes in the lung. Exposure to elevated concentrations of O_2 causes damage to cells of the bronchiolar and alveolar epithelium. The principal bronchiolar cell damaged by O_2 is the ciliated cell (Ludwin *et al.*, 1974). In the alveoli, type 1 cells and endothelial cells are the main cell types damaged (Pratt, 1974). Death of animals exposed to O_2 is usually associated with pulmonary edema. The mechanisms for repair of injured pulmonary tissues have only recently been described. In the bronchioles it was shown that nonciliated cells may divide and become ciliated cells following injury to ciliated cells (Evans *et al.*, 1977). In the alveoli, repair of damaged type 1 cell epithelium occurs through proliferation of type 2 cells and their subsequent differentiation into type 1 cells (Evans *et al.*, 1973, 1975; Adamson and Bowden, 1974). The mechanism for repair of damaged endothelium has not been described; however, it probably occurs through proliferation of endothelial cells (Bowden and Adamson, 1974). Thus, inhibition of cell division in the lung would also inhibit the repair process.

Although cell division is inhibited in the lungs of animals exposed to O_2, it is not known whether this is a direct effect of O_2 on the cells or an indirect one. Studies *in vitro* have shown that a variety of metabolic processes, including DNA synthesis, are inhibited during exposure to O_2, indicating that O_2 can act directly on the cell (Haugaard, 1968). However, in the intact animal, other factors such as increased stress hormones and starvation also decrease cell division (Bullough, 1965; Stirling *et al.*, 1973). In animals exposed to O_2, the adrenocortical response increases progressively in parallel with the severity of pulmonary O_2 toxicity (Clark and Lambertsen, 1971). Also, it has been noted (Leon *et al.*, 1971) that animals exposed to high O_2 eat less and lose weight; thus, both increased adrenocortical responses and decreased food intake could be factors associated with the depressed rate of cell division during exposure to O_2. The purpose of the present study was to determine whether decreased food intake is associated with a decrease in cell division. If so, this would suggest that supplementary nutritional intervention could modify or delay some of the adverse effects of breathing high oxygen.

METHODS

Previously described animal chambers were used for these studies (Hackney *et al.*, 1975). Temperature and humidity were monitored within both control and exposure chambers. Oxygen concentration within the exposure chamber was monitored with a Beckman E-2 O_2 analyzer. Oxygen was introduced into the exposure chamber from two manifolds, each with a flow rate of approximately 15 liter/min. Compressed room air was introduced into the other chamber. The daily environmental conditions during both experiments and in both control and exposure chambers were similar (mean ± SE): Temperature was 74 ± 3°F and relative humidity was 57 ± 4%. The average daily O_2 concentration in the exposure chamber during both experiments was 94 ± 1%.

Experiment 1—Cell Division

Seventy two Swiss-Webster mice (Charles River, Wilmington, Mass.), approximately 3 months old and pathogen free, were divided into two groups of

36. Thirty six of these animals were subdivided into two groups of 18 each, those with food and water and those with water only. Both groups of 18 were then exposed to a high concentration of O_2 for 5 days. The remaining 36 animals were subdivided into two groups as just described, placed within a similar chamber, and exposed to room air for 5 days.

Three animals from each of the four groups, high O_2 exposed (O), deprived of food and exposed to high O_2 (OD), control air breathing (A), and food deprived, air breathing (D), were sacrificed at 0, 1, 2, 3, 4, and 5 days. One hour before sacrifice, the mice were removed from the chambers, injected intraperitoneally with 100 μCi of tritiated thymidine ([^{3}H]TdR; sp act, 6.7 Ci/mmole; Biochemical and Nuclear Corp.), and returned to the chambers. The animals were sacrificed by cervical separation 1 hr after injection; the lungs were cannulated and instilled with 1% osmium tetroxide (OsO_4), buffered at pH 7.4 with Veronal–acetate buffer with 7.5% sucrose. The trachea was then ligated, and the lungs were removed and placed in a vial of buffered OsO_4 for 45 min. The tissue was sliced longitudinally and washed in three changes of Veronal–acetate buffer. After washing, the tissue was dehydrated in a graded series of ethanol–water mixtures and then passed through propylene oxide to a mixture of Araldite–propylene oxide, after which the tissue was infiltrated for 24 hr in Araldite.

TABLE I

Total Labeling Indexes

Time (days)	Air-breathing control group A (i)[a]		Air-breathing deprived group D (ii)		Oxygen-exposed group O (ii)		Oxygen-exposed deprived group OD (iii)	
	Animal number	TLI/1000[b]	Animal number	TLI/1000	Animal number	TLI/1000	Animal number	TLI/1000
0	140	14.0 (14.5)[c]	142	20.5 (13.9)	144	16.2 (14.5)	143	22.3 (16.1)
	144	16.3	146	11.2	145	NL[d]	147	11.6
	148	13.3	150	10.0	149	12.7	151	14.5
1	152	11.4 (11.4)	154	— (12.1)	153	4.5 (8.0)	155	25.5 (13.5)
	156	NL	158	11.1	157	10.0	159	3.0
	160	11.1	162	13.0	161	9.6	163	12.0
2	164	12.8 (13.9)	166	NL (4.0)	165	3.2 (6.4)	167	0 (0.7)
	168	20.0	170	3.0	169	—	171	1.0
	172	8.8	174	5.0	173	9.6	175	1.0
3	176	18.0 (14.9)	178	4.5 (2.7)	177	1.6 (4.4)	179	D[e] (0.3)
	180	13.3	182	2.5	181	5.0	183	0
	184	13.5	186	1.0	185	6.7	187	0.5
4	188	14.0 (14.3)	190	2.8 (4.7)	189	9.5 (5.9)	191	0.5
	192	11.9	194	10.8	193	1.1	195	D
	196	17.0	198	0.5	197	7.0	199	D
5	200	15.4 (17.2)	202	1.0 (1.7)	201	NL	203	D
	204	25.5	206	0.5	205	1.5	207	D
	208	10.6	210	3.5	209	D	211	D

[a] Lower-case Roman letters within parentheses indicate significant differences between groups. Groups labeled with the same letter are not significantly different, e.g., ii and ii. Groups labeled with different letters are significantly different ($P < 0.05$), e.g., i, ii, and iii.

[b] TLI/1000, total labeling index per 1000 cells counted.

[c] Mean values are given in parentheses.

[d] NL, not labeled.

[e] D, dead.

TABLE II

Food Consumption

Group	Δ Weight (gm)[a]					
	Day 1	Day 2	Day 3	Day 4	Day 5	Total
A	88.8	84.2	77.0	65.2	70.2	385.4
O	58.3	33.2	17.3	12.6	4.0[b]	125.4

[a] $N = 18$ except where indicated otherwise.
[b] $N = 13$.

After complete infiltration, the tissue slices were removed from the Araldite and placed on a large glass slide under a dissecting microscope, where selected units containing a terminal bronchiole, a respiratory bronchiole, and an alveolar duct were dissected out and embedded flat in Beem capsules, size 00. The capsules were polymerized in an oven at 60°C for approximately 17 hr.

One-micron sections were cut and prepared for light microscopic autoradiography. After 6 weeks of exposure, the autoradiographs were developed and stained with toluidine blue. The sections were studied under the light microscope, and approximately 3000 cells were counted from each animal. Examples of labeled cells and of the areas of tissue counted have been presented in other papers (Evans *et al.*, 1972, 1973). The number of labeled type 2 cells, alveolar macrophages, and cells in the alveolar wall (this last group made up mainly of leukocytes in the capillaries and endothelial cells) were determined. The labeling index (LI) was presented as labeled cells per 1000 alveolar cells counted (Evans *et al.*, 1972).

Experiment 2—Weight Loss

The grouping process was like that in experiment 1. Seventy two Swiss–Webster mice (Charles River), approximately 3 months old and pathogen free, were divided into two groups of 36. Thirty six of these animals were subdivided into two groups of 18 each, those with food and water and those with water only. Both groups of 18 were then exposed to a high concentration of O_2

TABLE III

Water Consumption

Group	Δ Weight (gm)[a]					
	Day 1	Day 2	Day 3	Day 4	Day 5	Total
A	126.4	127.1	115.6	104.2	102.6	575.9
D	117.1	110.4	80.5[b]	80.4[b]	54.6[c]	443.0
O	95.4	52.3	42.2	31.1	11.0[d]	232.0
OD	72.3	29.7	23.9	8.6	—	134.5

[a] $N = 18$ except where indicated otherwise.
[b] $N = 16$.
[c] $N = 14$.
[d] $N = 13$.

for 5 days. The remaining 36 animals were subdivided into two groups, as described above, placed in a similar chamber, and exposed to room air.

At the same time each day, each of the animals was placed in a beaker and carefully weighed on a Sartorius Kilomat III precision balance. Likewise, all food and water were weighed daily and recorded. As food and water levels decreased, the supply was replenished and weighed. At the conclusion of the 5-day exposure period, each animal was weighed and sacrificed, and the lungs were removed for examination. The remaining food and water were also weighed.

During the experiments, some animals died spontaneously and these are indicated in Tables I, II, and III.

RESULTS

General Observations

Control (A) and food-deprived air-breathing mice (D) had normal-appearing lungs. In the mice exposed to high O_2 (O), the lungs became dark and hemorrhagic (liver-like) by the fifth day. In the mice deprived of food and exposed to high O_2 (OD), the lungs were dark and hemorrhagic (liver-like) by the fourth day. In addition, these mice were thin and several had died by the fourth day.

Labeling Indexes

The LIs for the four groups of mice are presented in Table I and summarized in Fig. 1. The total LIs are presented because the main effect was decreased labeling and no differences in response were seen among the different cell types. On Day 0 (see Fig. 1) groups A, D, and O all had about the same LI; group OD had a slightly higher mean. On the first day of exposure all four groups exhibited a decrease in labeled cells per 1000. On succeeding exposure days the LI of group A appeared to increase, whereas that of the other groups decreased on the average.

In order to compare the trends of the four groups to locate any significant differences, linear regression analyses were performed. The LI trend of the

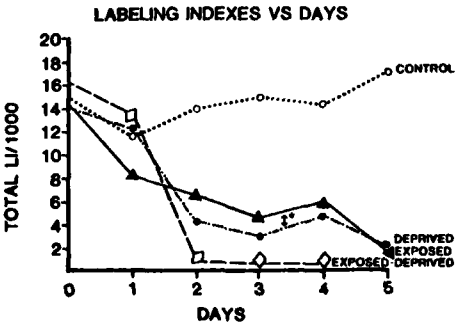

FIG. 1. Mean total labeling indexes from groups of mice studied for up to 5 days. Control (A), O · · · O; food deprived (D), ●–·–·–·–●; 100% oxygen exposed (O), ▲——▲; 100% O_2 exposed and food deprived (OD), ◇– – –◇. Asterisk indicates that groups O and D are statistically similar.

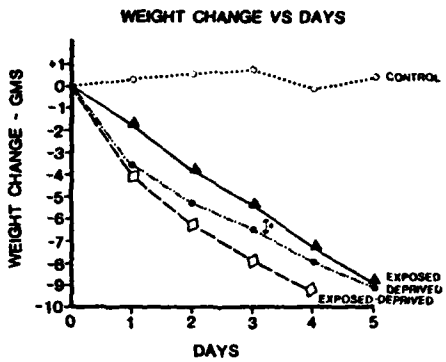

FIG. 2. Mean cumulative weight change (gm) of groups of mice studied for up to 5 days. Symbols are same as for Fig. 1.

control group (A) was significantly different from that of all other groups ($P < 0.001$). Groups were compared to the OD group on Days 0 through 4, since by the fifth day all OD animals had died. The trends of groups O and OD were statistically different ($P < 0.025$), whereas the trends of the deprived groups D and OD were marginally statistically different ($0.05 < P < 0.1$). Linear trend analysis of pooled data from statistically similar groups O and D compared with data from group OD was significantly different ($P < 0.05$).

Cumulative Weight Loss

A decrease in food intake will depress DNA synthesis in the tissues of mice. Since mice exposed to high O_2 tend to eat less, it is possible that the decreases in cell labeling observed during exposure to high O_2 were also caused by a lack of food. To help clarify the results from the labeling experiment, another experiment was undertaken to determine the weight loss in mice deprived of food and in those exposed to high O_2. The animals were weighed each day for 5 days and the average cumulative weight loss was determined. These results are summarized in Fig. 2. Control mice (A) maintained about the same weight through the 5 days studied. The other three groups exhibited an approximately linear decrease in weight loss over the 5-day period. In order to test for differences of cumulative weight loss between the four groups and between the consecutive days, a two-factor, repeated-measures analysis of variance was performed. Since the groups and the days were each significantly different at the 0.05 level, the Newman–Keuls contrast procedure was performed on all pairwise comparisons. Group O was not significantly different from group D in mean cumulative weight losses. All other pairwise group comparisons were different at the 0.05 level. In regard to daily mean cumulative weight loss, there was a significant decrease in weight from one day to the next when averaging over all groups.

Food consumption for the control group (A) and group O is presented in Table II. During the 5 days of testing, the control group (A) had a slight decrease in the amount of food consumed. In group O, a 34.4% decrease in food intake occurred on the first day of exposure. Decreased food intake continued so that by the fifth day it was 92.1% (corrected for the number of animals

remaining in the two groups by the fifth day) less than that consumed by the controls. Water consumption decreased consistently in all three experimental groups (Table III). The greatest decrease was in the two groups exposed to high O_2. Water was equally available to all groups.

DISCUSSION

In the present study, cell division (as measured by [^{3}H]TdR labeling indexes), weight loss, and food and water consumption were compared between groups of mice exposed to: air (A), air and deprived of food (D), high O_2 (O), high O_2 and deprived of food (OD). Groups D and O showed statistically similar progressive decreases in cell labeling and weight loss. Group OD had even greater decreases. Mice exposed to high O_2 had a decreased rate of food intake. These results show that:

Complete food deprivation with weight loss causes a decrease in the number of cells synthesizing DNA.

Exposure to high O_2 causes a decrease in DNA synthesis, similar to that seen with food deprivation.

Exposure to high O_2 results in a decreased food intake and produces about the same weight loss as complete food deprivation.

Complete food deprivation of high-O_2-exposed mice produces more weight loss and decreases DNA synthesis even more.

Thus, to the extent that it occurs a decreased-food-associated weight loss during exposure to high O_2 is a principal factor in decreasing DNA synthesis.

The role that decreased food intake has in decreasing DNA synthesis may have many facets. Stirling *et al.* (1973) showed that food deprivation inhibited cell proliferation in regenerating liver. A few of the possible mechanisms were: (1) deficiency of RNA and protein synthesis, (2) decreased caloric intake, and (3) activation of stress pathways. In their studies, they concluded that protein deficiency was the most important inhibitory effect from food deprivation.

In the lung, Gacad *et al.* (1972) showed that food deprivation caused a decrease in protein synthesis. Since mice exposed to O_2 have a decreased food intake and concurrent weight loss, it may be concluded that they also have a decrease in protein synthesis. In support of this concept, Bieber *et al.* (1971) showed that ribosome synthesis (and presumably protein synthesis also) was affected in newborn guinea pig lung exposed to 100% O_2. Since protein synthesis is necessary for DNA synthesis, its decrease during exposure to 100% O_2 may be the principal factor causing the decrease in DNA synthesis.

The reason mice deprived of food and exposed to O_2 had a greater decrease in DNA synthesis than mice in either group alone is probably the direct O_2-inhibitory action on the cell as mentioned earlier. Since the weight changes in O_2-exposed and food-deprived unexposed mice were about the same, but the O_2-exposed mice continued to consume some food and water, the relative contribution of O_2 exposure versus food deprivation in this group (O) cannot be quantitated.

The reason for decreased food intake during exposure to high O_2 is not known. Besides the above-mentioned effects on decreasing DNA synthesis, another effect may be nutritional deficiencies in the cells (antioxidant levels)

resulting in a decreased ability of the cells to withstand injury by O_2. The time course of O_2-induced injury to lung cells supports this concept. Visible injury to lung cells is not observed until the third day of exposure. The present study shows that by this time there is a large decrease in the amount of food consumed, and, presumably, also in dietary antioxidants. Supplementation of dietary antioxidants before exposure to O_2 has been shown to afford some protection against O_2 toxicity (Schatte and Swansinger, 1976). However, additional studies of enforced food intake during exposure to O_2 are needed to clarify this question.

Also, if animals survive the initial 5 days of exposure, proliferation of cells begins and the animals will probably survive. Although Northway *et al.* (1976) reported a lack of weight gain in paired litter-mate newborn mice exposed to 100% O_2, and they suggested that this was probably due to diminished food intake, weight change or food consumption have not usually been documented in studies of this sort so their roles are not known. However, proliferation following cellular injury by O_2 occurs in the same manner as that reported for ozone and nitrogen dioxide. This suggests that either the stimulus for repair-initiated proliferation is strong under these conditions or improved food consumption occurs. It is possible that both are involved. Forced-feeding studies are currently underway in this laboratory to help clarify these last questions.

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6

EFFECT OF HIGH CONCENTRATIONS OF OXYGEN ON REPARATIVE REGENERATION OF DAMAGED ALVEOLAR EPITHELIUM IN MICE

EFFECT OF HIGH CONCENTRATIONS OF OXYGEN ON REPARATIVE REGENERATION OF DAMAGED ALVEOLAR EPITHELIUM IN MICE

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ABSTRACT

The main purpose of the present study was to determine if high concentrations of oxygen (O_2) would inhibit cell division in alveoli undergoing reparative regeneration following injury by ozone (O_3). Mice were exposed to 2.5 ppm O_3 for six hours to injure the alveolar epithelium. Groups of animals were then allowed to recover in 20% O_2 with and without food, 40%, 61%, 80%, and 95% O_2 with food. At daily intervals, the mice were injected with tritiated thymidine to label cells preparing to divide, and sacrificed one hour later. Lung tissue was prepared for light microscopic autoradiography and the proportion of labeled cells determined. In mice exposed to O_3, type 1 epithelium is injured and type 2 cells proliferate in order to repair the damage. Mice allowed to recover in 20% O_2 with and without food, and in 40% O_2, had a maximum proliferation of type 2 cells (about eight times control) on the second day of recovery. By the fourth day, repair seemed to be complete and labeling indexes had returned to normal. In mice allowed to recover in 61%, 80%, and 95% O_2, type 2 cell proliferation was less than control on the second day and did not increase during four days of recovery. Electron microscopy of the tissue showed type 1 epithelium had not been replaced. It was concluded that concentrations of O_2 at 61% and higher inhibit reparative cell division in O_3-damaged alveoli of mice.

INTRODUCTION

During the first day of exposure to high concentrations of oxygen (O_2) the normal rate of cell division in the lung is decreased (1). In animals that survive the initial exposure to O_2 or in those exposed to lower concentrations, cell division resumes after about five days (2-5). Although cell division is inhibited in the lungs of animals exposed to O_2, it is not known whether this is a direct effect of O_2 or an indirect one. Studies *in vitro* have shown that a variety of metabolic processes, including DNA synthesis, are inhibited during exposure to O_2, indicating that O_2 can act directly on the cell (6). However, in the intact animal, other factors such as increased stress hormones and starvation also decrease cell division (7,8). In animals exposed to O_2, it has been noted that they eat less and lose weight (9). Recently Hackney et al. (10) showed that air-breathing mice deprived of food had weight loss and depression in DNA synthesis similar to that in mice exposed to 100 percent O_2. This indicates that indirect factors are associated with the depression of DNA synthesis observed in mice exposed to high O_2.

In animals that survive exposure to high concentrations of O_2, proliferation of cells begins and the tissue is repaired (4). This suggests that either the stimulus for repair-initiated proliferation is strong, improved food consumption occurs, or the cells adapt in some way to the O_2. Recently Witschi and Cote (11) showed in mouse lung damaged by butylated hydroxytoluene (BHT) that DNA synthesis for repair was inhibited by high concentrations of O_2. In this study DNA synthesis was measured biochemically and it was not determined which cell types were involved. Because weight changes were not taken, it was not known if food consumption was a factor in the decreased rate of DNA synthesis. The purpose of the present study was to determine: (1) if Type 2 cell proliferation induced by ozone (O_3) injury of the lung could be inhibited by high O_2, and (2) if decreased food consumption was a factor.

METHODS

Previously described exposure facilities were used for these studies (5). Temperature and humidity were monitored within both control and exposure chambers. Ozone was generated from dried filtered air by high voltage discharge. The concentration was determined by ultraviolet radiation absorption (Dasibi 1003-AH Ozone Meter) calibrated according to the neutral buffered potassium iodide method. Oxygen concentration within the chambers was monitored by an oxygen analyzer (S-3A Applied Electrochemistry Inc.).

A total of 104 Swiss-Webster male mice (Charles River, Wilmington, Mass.) approximately three months of age and pathogen free were used for this study. Initially four animals were selected at random and sacrificed for quality control (zero day pre O_3 control), while the remaining animals were weighed and randomly placed into one of six groups. The mean and standard deviation for frequent measurement of percent O_2 during the experiment are given in parentheses following the designation for each group--Group I: Room Air Control (19.8 ± 0.4), Group II: Room Air Deprived (19.8 ± 0.4), Group III: 40 percent oxygen (39.5 ± 1.4), Group IV: 61 percent oxygen (60.8 ± 1.5), Group V: 80 percent oxygen (80.9 ± 3.3), Group VI: 95 percent oxygen (94.7 ± 0.9). Group I consisted of 20 animals and the remaining five groups consisted of 16 animals each. All groups were exposed to 2.5 ppm O_3 for six hours, after which they were removed from the O_3 and placed within their respective exposure chambers for the remaining four days. Four animals from Group I were sacrificed immediately post ozone exposure (zero day post O_3 control). At daily intervals four mice from each group were injected with tritiated thymidine (^{3}H-TdR) to label cells preparing to divide, and sacrificed one hour later by an overdose of Sodium Pentobarbital injected peritoneally; the lungs were cannulated and infiltrated with 2 percent Gluteraldehyde. The trachea was then ligated and the lungs were removed. After one hour's fixation the lungs were washed in

Sodium Cacodylate buffer, sliced, rinsed in buffer, then placed in a vial of buffered OsO_4 for 45 minutes. The tissue was then washed in three changes of Sodium Cacodylate buffer. After washing, the tissue was dehydrated in a graded series of ethanol-water mixtures and then passed through propylene oxide to a mixture of Epon-Araldite-propylene oxide, after which the tissue was infiltrated for 24 hours in Epon-Araldite (Anderson's mixture).

After complete infiltration, the tissue slices were removed from the Epon-Araldite and placed on a large glass slide under a dissecting microscope where selected units containing a terminal bronchiole, a respiratory bronchiole, and an alveolar duct were dissected out and embedded flat in Beem capsules, size 00. The capsules were polymerized in an oven at $60^{o}C$ for approximately 48 hours.

One-micron sections were cut and prepared for light microscopic autoradiography. After six weeks of exposure, the autoradiographs were developed and stained with toluidine blue. The sections were studied under the light microscope, and approximately 2000 cells were counted from each animal. Examples of labeled cells and of the areas of tissue counted have been presented in other papers (12, 13). The number of labeled type 2 cells, alveolar macrophages, and cells in the alveolar wall (this last group made up mainly of leukocytes in the capillaries and endothelial cells) were determined. The labeling index (LI) was presented as labeled cells per 1000 alveolar cells counted (12).

Just before sacrifice, at the same time each day, each of the animals was placed on a Onaus Triple Beam Animal Balance and carefully weighed. Likewise, all food and water were weighed daily and recorded. As food and water levels decreased, the supply was replenished and weighed.

RESULTS

General Observations

Control and O_3 exposed mice recovering in 20 percent O_2 with and without food and those recovering in 40 percent O_2 with food had normal appearing lungs. In mice exposed to O_3 and recovering in 95 percent O_2, the lungs had become dark and hemorrhage by the third day.

Labeling Indexes

The LIs of type 2 cells, alveolar macrophages, and remaining cell types for the six groups of mice are presented in Table 1. Type 2 cell labeling indexes represent the ability to repair damaged type 1 epithelium (13,14). In experimental groups I - III, the type 2 cell LI reached a peak on the second day of recovery and declined to control levels on the third and fourth day (Fig. 1). This represents a typical response for repair of damaged type 1 epithelium (15). In groups IV - VI, the type 2 cell labeling index was depressed throughout the four days of recovery, indicating that proliferation for repair of type 1 cell damage had not occurred (Fig. 1).

Alveolar macrophage labeling indexes were similar to controls in groups I and VI during the four days of recovery. In the remaining experiments the LI was elevated above controls after one day of recovery but were significantly different from controls only in group IV. In all groups the labeling indexes were similar to controls from the second to fourth day of recovery.

The labeling index of cells in the alveolar walls represent all other cell types in the alveoli that have incorporated ^{3}H-TdR. The patterns of labeling indexes during the four days of recovery were generally similar among the exposure and control groups.

Weight Change

The weight change for animals in groups I - VI are summarized in Figure 2. In groups I, III - V there was no change from controls. In groups II and VI, a steady weight loss occurred that was significantly different from controls and days one - four for group II, and two - four for group VI.

DISCUSSION

In the alveoli of mice exposed to low concentrations of O_3, damage is confined mainly to type 1 epithelium (15). Following the injury, type 2 cells proliferate and differentiate into type 1 cells to repair the damaged epithelium. Proliferation of other cells also occurs but its significance is not completely understood. This discussion will deal only with proliferation of type 2 cells.

The proliferative response of type 2 cells in mice exposed to O_3 and allowed to recover in 20 percent oxygen with food was similar to that reported previously (15). Mice recovering from O_3 injury in 20 percent oxygen without food and those recovering in 40 percent oxygen with food also had a normal type 2 cell proliferative response. These data indicate that lack of food or 40 percent oxygen did not inhibit type 2 cell division associated with repair of damaged type 1 epithelium.

In mice recovering from O_3 injury in 61, 80, and 95 percent oxygen, type 2 cell proliferation was inhibited during the four days of recovery. In mice recovering in 95 percent O_2 there was a weight loss comparable to mice deprived of food, confirming a previous observation (10). Mice recovering in 61 and 80 percent oxygen exhibited no weight changes. Because 61 and 80 percent oxygen inhibit type 2 cell division without weight loss, and in mice deprived of food and exhibiting a weight loss there was no such inhibition, we conclude that oxygen per se in the concentrations of 61, 80, and 95 percent acts on type 2 cells to inhibit cell division.

The results obtained here are similar to the findings of Witchi and Cote (11). They reported inhibition of total lung DNA synthesis in mice injured by butylated hydroxytoluene (BHT) and allowed to recover in 60, 80 and 100 percent oxygen. In mice recovering in 40 percent oxygen DNA synthesis was not significantly inhibited. Our observations confirm their tentative conclusion that dividing epithelial cells are sensitive to the toxic effects of oxygen.

The results obtained by ourselves and those of Witchi and Cote (11) may be of practical importance. It is clear that concentrations of oxygen as low as 60 percent could inhibit the repair processes in the lung following acute lung injury. This could lead to lethal pulmonary edema such as that demonstrated in aging rats exposed to NO_2 in which the onset of type 2 cell proliferation was delayed one day (16). Also it has been suggested that inhibition of epithelial cell division associated with repair may lead to fibrosis (17). A large number of toxic agents cause injury to type 1 epithelium followed by type 2 cell proliferation (18). Understanding factors associated with control of reparative cell proliferation in the lung, particularly the identification of agents that inhibit this process, would be of importance in the treatment of such injury.

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Table 1

LABELING INDEXES FROM MICE EXPOSED TO OZONE AND ALLOWED TO RECOVER UNDER VARIOUS CONDITIONS

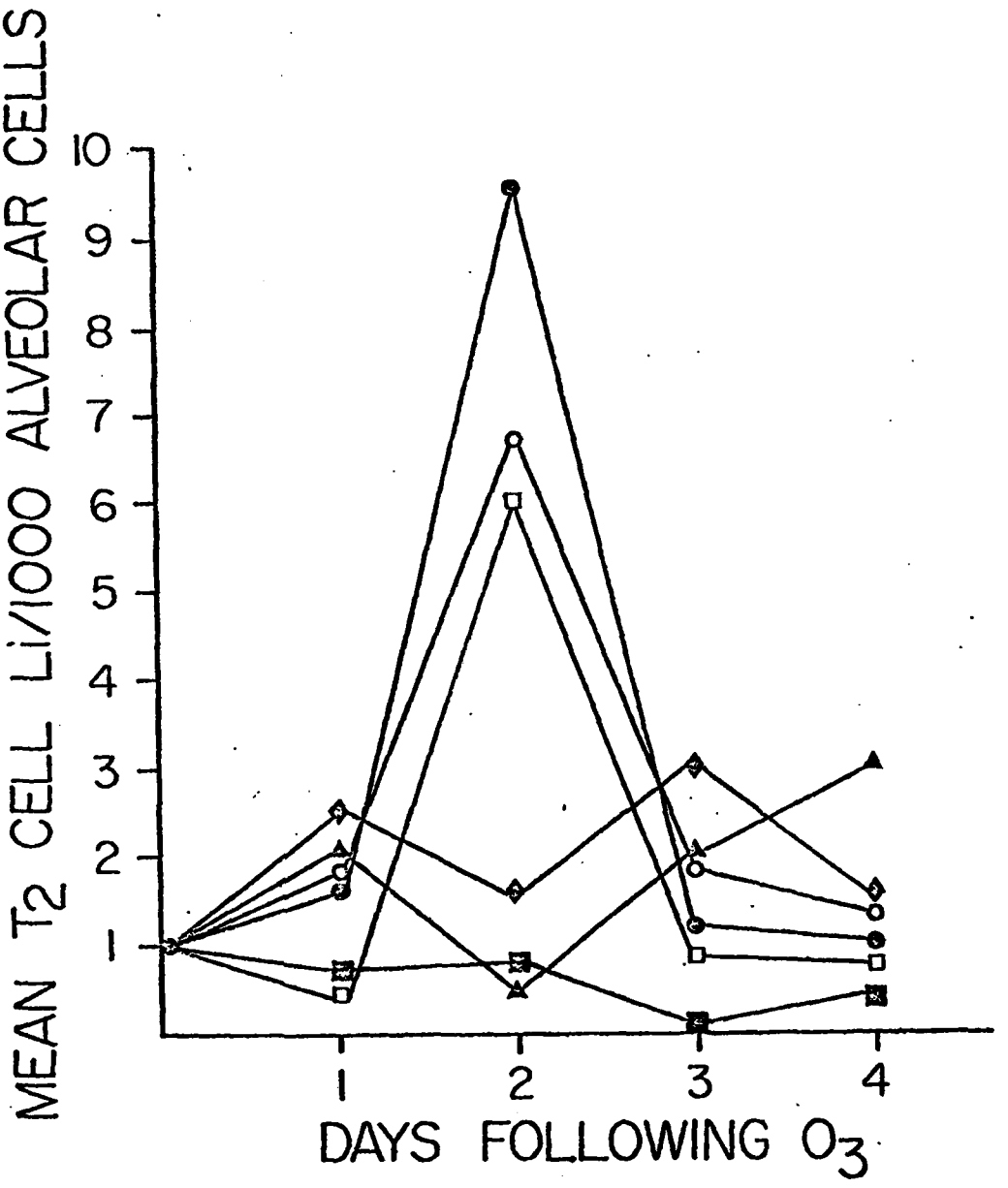
EXPERIMENTAL GROUP	RECOVERY DAY	LABELED CELLS PER 1000 ALVEOLAR CELLS: TYPE 2 CELLS	ALVEOLAR MACROPHAGES	CELLS IN THE ALVEOLAR WALLS
I	1	1.5 ± 1.0	2.0 ± 1.4	9.5 ± 3.7
20% O_2	2	9.3 ± 5.7	2.3 ± 1.0	12.8 ± 2.5
+ Food	3	.8 ± .5	1.3 ± .5	10.8 ± 10.5
	4	1.0 ± 1.0	.8 ± 1.1	9.5 ± 2.6
II	1	.5 ± .5	6.3 ± 5.2	7.3 ± 2.9
20% O_2	2	6.0 ± 3.6	2.3 ± 1.9	12.0 ± 6.3
- Food	3	.5 ± .5	.5 ± .5	6.8 ± 3.9
	4	.8 ± .5	.3 ± .5	8.0 ± 3.6
III	1	1.8 ± 2.1	5.8 ± 3.8	6.5 ± 2.6
40% O_2	2	6.8 ± 2.2	3.0 ± 1.0	8.3 ± 3.0
	3	1.7 ± 2.0	2.0 ± 0	8.7 ± 7.2
	4	1.3 ± .5	1.5 ± 2.0	10.5 ± 2.1
IV	1	2.5 ± 2.3	9.8 ± 2.3	9.3 ± 4.4
61% O_2	2	1.5 ± 1.2	3.0 ± 2.7	13.6 ± 5.2
	3	3.0 ± 2.4	1.5 ± 1.2	20.1 ± 9.1
	4	1.5 ± 1.2	2.3 ± 2.6	11.5 ± 6.6
V	1	2.0 ± 1.6	7.3 ± 7.8	7.8 ± 4.1
80% O_2	2	.3 ± .4	1.5 ± 1.2	4.3 ± 3.9
	3	1.8 ± 1.5	2.8 ± 2.2	4.8 ± 3.1
	4	3.0 ± 2.6	.5 ± 1.0	9.0 ± 5.0

Table 1 (Continued)

LABELING INDEXES FROM MICE EXPOSED TO OZONE AND ALLOWED TO RECOVER UNDER VARIOUS CONDITIONS

EXPERIMENTAL GROUP	RECOVERY DAY	LABELED CELLS PER 1000 ALVEOLAR CELLS: TYPE 2 CELLS	ALVEOLAR MACROPHAGES	CELLS IN THE ALVEOLAR WALLS
VI	1	.8 ± .9	2.5 ± 1.9	6.5 ± 4.5
95% O_2	2	.8 ± .9	1.0 ± .8	5.0 ± 1.4
	3	0	0	5.5 ± 3.3
	4	.3 ± .4	.5 ± 1.0	1.5 ± .6
(0 DAY - PRE O_3)		1.0 ± 2.0	1.5 ± 1.7	7.3 ± 3.8
(0 DAY - POST O_3)		1.3 ± .5	1.5 ± 2.4	9.3 ± 8.2

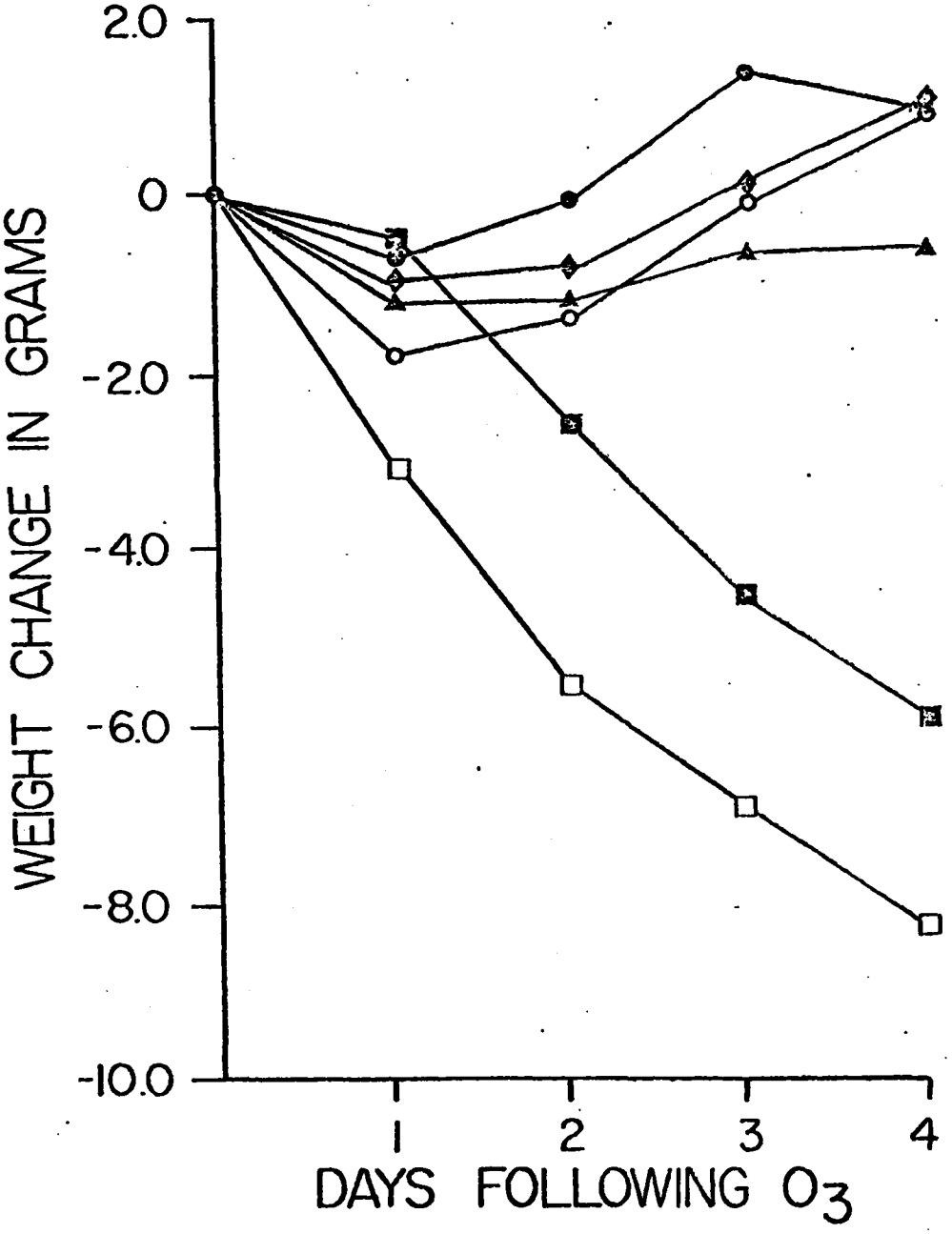
FIGURE 1



TYPE 2 CELL LABELING INDEXES FROM MICE EXPOSED TO O_3 AND ALLOWED TO RECOVER UNDER VARIOUS CONDITIONS:

- Group I (20% O_2 with food)
- Group II (20% O_2 without food)
- Group III (40% O_2 with food)
- Group IV (61% O_2 with food)
- Group V (80% O_2 with food)
- Group VI (95% O_2 with food)

FIGURE 2



WEIGHT CHANGES IN MICE FROM GROUPS I - VI FOLLOWING INJURY BY O_3:

- Group I (20% O_2 with food)
- Group II (20% O_2 without food)
- Group III (40% O_2 with food)
- Group IV (61% O_2 with food)
- Group V (80% O_2 with food)
- Group VI (95 % O_2 with food)